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Report

Cytogenetic Biodosimetry in Radiation Emergency Medicine: 1. Blood Collection and Its Management

Yohei Fujishima[1], Yu Abe[2], Valerie Goh Swee Ting[3], Ryo Nakayama[1, 4], Kai Takebayashi[1, 4], Akifumi Nakata[5], Kentaro Ariyoshi[6], Mai Tran Thanh[7], Kosuke Kasai[4], Hiroyuki Hanada[8], Mitsuaki A. Yoshida[1, 9], Katsuhiro Ito[8]* and Tomisato Miura[1]**

[1]*Department of Risk Analysis and Biodosimetry, Institute of Radiation Emergency Medicine, Hirosaki University, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan*
[2]*Department of Radiation Biology and Protection, Atomic Bomb Disease Institute, Nagasaki University, 1-12-4 Sakamoto, Nagasaki, Nagasaki 852-8523, Japan*
[3]*Department of Radiobiology, Singapore Nuclear Research and Safety Initiative, National University of Singapore, 1 Create Way, Singapore 138602, Singapore*
[4]*Department of Bioscience and Laboratory Medicine, Hirosaki University Graduate School of Health Sciences, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan*
[5]*Faculty of Pharmaceutical Sciences, Hokkaido University of Science, 15-4-1, Maeda 7-jo, Teine-ku, Sapporo, Hokkaido 006-8585, Japan*
[6]*Center for Integrated Science and Humanities, Fukushima Medical University, 1 Hikariga-oka, Fukushima City, Fukushima, 960-1295, Japan*
[7]*Biodosimetry Group, Centre of Radiation Technology and Biotechnology, Dalat Nuclear Research Institute, 1 Nguyen Tu Luc, Ward 8, Dalat City, Lamdong Province, Vietnam*
[8]*Advanced Emergency and Critical Care Center, Hirosaki University Hospital, Hirosaki University, 53 Hon-cho, Hirosaki, Aomori 036-8563, Japan*
[9]*Institute of Chromosome Life Science, 11-5-409, Fukuokachuo 2-Chome, Fujimino-shi, Saitama 356-0031, Japan*

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Dose assessment is very important to triage exposed patients and to carry out efficient medical care and treatment in radiation emergency medicine. In cytogenetic biodosimetry, peripheral blood collected from exposed patients must be cultured to induce chromosome-analyzable metaphases in peripheral lymphocytes. Medical institutions that accept exposed patients must understand the time of blood sampling, choice of anticoagulant, temperature conditions for blood storage according to the type of anticoagulant and the method of blood transportation to the laboratory for biodosimetry. However, as medical institutions tend to have insufficient understanding on blood collection and shipment required for biodosimetry, this information must be provided to aid in reliable dose estimation. In addition, dose assessment requires some basic information from patients such as age, gender, smoking history, alcohol intake, underlying medical conditions and previous radiation exposures including occupational and medical exposure. The medical institution should also be prepared to provide such information to the biodosimetry laboratory. This article provides a summary of essential information from blood collection to blood transportation carried out by medical institutions for cytogenetic biodosimetry.

Key words: cytogenetic biodosimetry, blood collection, timing, anticoagulant, storage, shipment

*Katsuhiro Ito: Advanced Emergency and Critical Care Center, Hirosaki University Hospital, Hirosaki University, 53 Hon-cho, Hirosaki, Aomori 036-8563, Japan
E-mail: itohkcck@hirosaki-u.ac.jp
**Tomisato Miura: Department of Risk Analysis and Biodosimetry, Institute of Radiation Emergency Medicine, Hirosaki University, 66-1 Hon-cho, Hirosaki, Aomori 036-8564, Japan
E-mail: tomisato@hirosaki-u.ac.jp

1. Introduction

In radiation emergency medicine, it is necessary to estimate radiation dose in order to triage exposed patients and to formulate a treatment plan. In dose assessment, the patient is first classified into roughly estimated dose categories based on the prodromal symptoms of the patient[1, 2]. Furthermore, the exposed dose can be estimated by physical methods if internal and/or external radioactive contamination occurs. However, in the case of external exposures by ionizing radiation such as X-rays or gamma rays, as there are no residual radioactive substances in the exposed patient, the absorbed dose is estimated based on biological reactions in the human body. This form of biological dose assessment is known as biodosimetry.

In addition to monitoring blood cell counts in peripheral blood of exposed patients[2, 3], biomarkers such as C-reactive protein (CRP) and salivary α-amylase (sAA) can also be used for biodosimetry[4]. However, as these endpoints show individual differences even without radiation exposure[5], accurate dose assessment can be difficult.

The most reliable endpoint in biodosimetry is the frequency of chromosome aberrations. In the process of repairing double-stranded DNA break induced by radiation, chromosome aberrations such as dicentric chromosomes, ring chromosomes and chromosome translocations can occur due to chromosome recombination errors[6]. As these chromosome aberration frequencies positively correlate with absorbed dose, absorbed dose can be easily calculated from a dose-response curve. The dicentric chromosome assay, which uses the frequency of dicentric chromosomes as an endpoint, has been the international gold standard for biodosimetry for about 70 years[7].

Cells used for chromosome analysis are generally peripheral blood lymphocytes, more specifically T cells. Chromosomes cannot be directly observed in lymphocytes after blood collection because lymphocytes do not undergo cell division in peripheral blood. Peripheral blood lymphocytes therefore require stimulation and culture under appropriate conditions to induce cell division for analyzable metaphase chromosomes. Please refer to other articles in the "Biodosimetry in Radiation Emergency Medicine" series for more information.

In order to culture blood for chromosome aberration analysis, peripheral blood is collected using an appropriate anticoagulant at an acceptable timing. Collected blood is stored under recommended conditions according to the type of anticoagulant used. The blood must then be sent to the biodosimetry laboratory as needed. Moreover, patient information is also required for reliable dose assessment. Communication between medical institutions handling radiation exposed patients and biodosimetry laboratories is thus essential. Details regarding blood collection and its management for biodosimetry involving medical institutions and biodosimetry laboratories can be found in this article.

2. Timing of blood collection

It is highly likely that the exposed patient will not be evenly exposed to external radiation (i.e. partial body radiation). Likewise, the circulating blood in the body will not be uniformly exposed. For example, if the patient was exposed to a radiation source on the right side, the absorption rate will differ depending on the radiation quality, but the absorbed dose on the left side will be lower than that on the right side. Tamura *et al.* collected peripheral blood from patients after gamma-ray therapy and analyzed a temporal change of the frequency of chromosome aberrations. Chromosome aberration-positive cells peaked after 6 hours. After 20 hours, chromosome aberration-positive cells reached the same level as 20 minutes after exposure[6, 8]. In biodosimetry, as whole-body exposure dose is estimated from a very small portion of circulating peripheral blood, the patient's blood is advised to be collected after the blood in the body is sufficiently mixed and homogeneous. For this reason, it is recommended to collect peripheral blood 24 hours after radiation exposure in radiation emergency medicine.

In addition, the dicentric chromosome found in exposed patients is an unstable chromosome abnormality. Its biological half-life is estimated to be 14 to 18 months[9, 10]. Therefore, the frequency of dicentric chromosomes gradually decreases with increasing time from the accidental exposure. Brewen *et al.* reported that unstable chromosome aberrations (dicentric and ring chromosomes) did not change in peripheral blood lymphocytes of patients who were systemically exposed to radiation accidents until 32 days after exposure[11]. Estimated doses derived from the reduced dicentric chromosome frequency will be lower than the original exposed dose, resulting in an underestimation of the patient's dose. Therefore, it is recommended that blood should be drawn from patients within 28 days of exposure for dicentric chromosome assay[6].

On the other hand, radiation-sensitive lymphocytes can immediately decrease in high-dose exposed patients. In the JCO Tokai-mura criticality accident, peripheral blood lymphocyte counts of the two workers who died from high-dose exposures were 684 cell/μl (3%) and 127 cells/μl (1%), respectively, 2–3 hours after the exposure[12]. Patients exposed to high doses may not have sufficient peripheral blood lymphocytes for chromosome aberration analysis after 24 hours. If high-dose exposure of 4–5 Gy or higher is suspected from prodromal symptoms, peripheral blood

for biodosimetry should be drawn as soon as possible.

For patients who require a blood transfusion, blood for biodosimetry must be collected prior to the transfusion. However, if the patient's condition is serious and it is determined that blood collection is difficult, blood collection is not necessary and should not be forced.

3. Acquisition of basic information and consent

In biodosimetry with chromosome aberration analysis, it is necessary to obtain information of exposed patients such as age, gender, smoking history, alcohol intake, medication intake, underlying medical conditions and previous radiation exposures such as radiotherapy, X-ray diagnosis and nuclear medicine[13]. An example of a questionnaire is shown in the Appendix. This basic information may affect the frequency of chromosome aberrations and dose estimation, depending on the type of chromosome abnormality used as an endpoint. It is necessary to record the information obtained from the exposed patient or his/her family in a standardized format and share it with the biodosimetry laboratory.

In addition, in order to learn from the mistakes of past radiation-related accidents, it is also important to disclose this information including estimated exposure doses. As the estimated dose is part of the diagnosis, the patient's and family's consent is required for information disclosure. It should be noted that in the case of an occupational accident, the consent of the employer is also required.

4. Anticoagulants

In specimen collection for clinical use, various anticoagulants and blood collection tubes are used depending on the examination purpose. The International Council for Hematology Standardization recommends ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for blood cell count measurement and blood cell morphology examination on blood smears[14]. On the other hand, in critical care, which requires rapid tests, blood is collected using heparin as an anticoagulant for blood gas test and electrolyte test, and blood cell count is measured using the leftover blood. These same blood tests are likely to be used in radiation emergency medicine. In addition, serum or plasma can also be used for biomarker measurement.

Heparin is the preferred anticoagulant in chromosome aberration analysis. Heparin does not have a direct anticoagulant effect, but it binds to the endogenous anticoagulant factor, antithrombin, to form a complex. This in turn enhances the activity of antithrombin, inhibits the activity of thrombin and blood coagulation factor X, and suppresses blood coagulation[15-17]. Low temperature conditions are not recommended for heparin blood storage because antithrombin activity decreases and partial blood coagulation occurs under low temperature conditions[18].

Heparin also allows lymphocyte proliferation to occur in peripheral blood cultures as it does not affect calcium ions necessary for cell division[19-22]. In contrast, EDTA chelates calcium ions in the culture medium required for lymphocyte proliferation. Lymphocyte cell division is unable to be effectively induced and thus affects the number of analyzable metaphases for biodosimetry (Fig. 1)[18]. If EDTA blood only is available for biodosimetry, the medical institution which collected the blood must inform the biodosimetry laboratory that EDTA was used as an anticoagulant.

5. Blood volume

The amount of blood used for chromosome aberration analysis depends on the culture method and the number of assays. For example, whole blood cultures use 0.5–1.2 ml of blood, whereas peripheral blood mononuclear cell (PBMC) cultures use about 3 ml of blood. More blood is required if various cytogenetic assays are performed together with the dicentric chromosome assay, such as the premature chromosome condensation (PCC) assay and the cytokinesis-block micronucleus (CBMN) assay. In addition, if the initial blood culture fails for some reason, the stored leftover blood is re-cultured. Considering these factors, it is thus recommended to collect about 10 ml of peripheral blood.

As heparin blood is used for blood gas test, electrolyte test and blood cell count measurement in critical care medicine, it is also possible to use the leftover blood after clinical analysis for biodosimetry. When storing leftover blood, bacterial contamination should be avoided as much as possible.

6. Blood storage

As described above, when heparin is used as an anticoagulant, low temperature conditions may cause partial blood coagulation due to incomplete coagulation (Fig. 2)[18]. When the number of lymphocytes required for blood culture decreases, a sufficient number of metaphases for chromosome analysis cannot be obtained. In exposed patients, blood clots may become larger and lymphocyte depletion may be even more pronounced. It is thus highly recommended to store heparin blood at 18–24 °C[23]. Blood temperature control in cold regions is also essential. Based on the authors' previous experiences, partial coagulation was seen in blood exposed to low ambient temperature (<5 °C) and made chromosome analysis difficult.

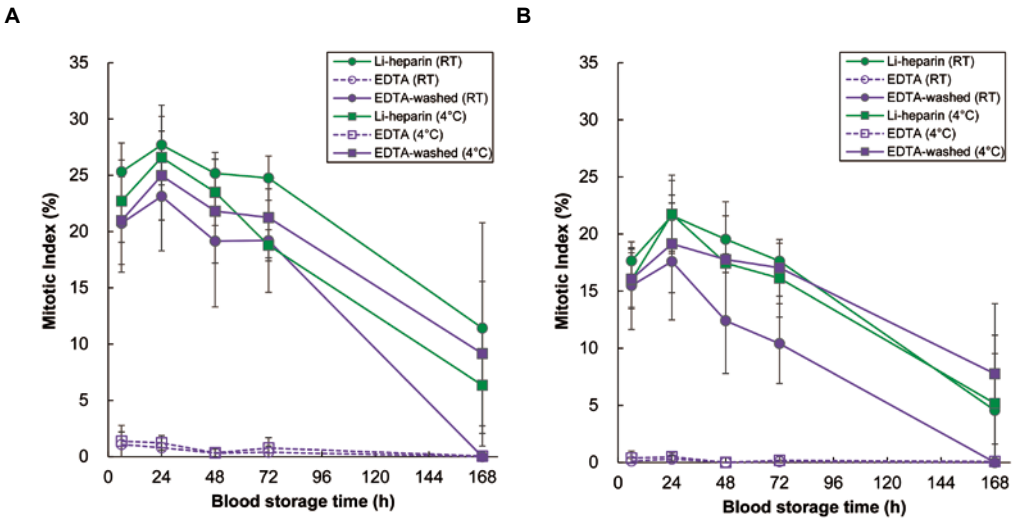

Fig. 1. Effect of temperature, storage time, anticoagulant type and radiation dose on mitotic index. Mitotic index was analyzed in blood cultured for 48 hours after storing them in different time periods and conditions. (A) 0 Gy; (B) 3 Gy of X-ray (150 kVp, 20 mA, with 0.5 mm Al, and 0.3 mm Cu filters, 1.0 Gy/min). Data is represented as Mean ± SD. (Data was modified from Fujishima *et al.*[18]).

On the other hand, EDTA blood can be stored from 4 ℃ to room temperature. Refrigerated storage is highly recommended if it is not used immediately after blood collection. When EDTA is used as an anticoagulant, even if blood is stored in a refrigerator at 4 ℃, blood coagulation is not affected[18, 23]. Furthermore, even when the EDTA blood stored in a refrigerator is cultured, the frequency of cell division was equivalent to that of heparin blood when EDTA was removed by washing before blood culture (Fig. 1B)[18].

It should also be noted that blood is an infectious specimen. Proper understanding of blood-borne infectious diseases and their countermeasures and appropriate blood storage procedures is advised.

7. Blood shipment

Transportation of blood obtained from exposed patients to biodosimetry laboratories requires special care in temperature control and handling of blood as an infectious specimen. In the case of heparin blood, it is necessary to maintain the temperature at 18 to 24 ℃ using temperature controlled packaging[6, 23]. When transporting EDTA blood, refrigerator packs can be added to maintain a low temperature. Temperatures should not be too low such that the blood becomes frozen. If possible, a temperature logger may be added in the shipment to record the temperature inside the packaging during the delivery.

In addition, human blood is classified as a UN standard category B biological substance. It must be shipped in triple packaging using Category B UN standard containers (UN3373) in accordance with WHO guidelines, "UN3373: Guidance on Transport Regulations for Infectious Substances"[24]. The primary receptacle is the closed blood collection tube. Next, the primary receptacle is wrapped in sufficient absorbent material and packed in a leak-proof secondary package. Lastly, it is placed in an outer container that protects against impact during transportation (Fig. 3). It is also advisable to use temperature controlled packaging that complies with UN and WHO standards.

If blood samples need to be transported to the biodosimetry lab via air, X-ray security checks must be avoided to prevent any unnecessary radiation exposure which could affect the true dose estimate. The outer packaging should be labelled with "Do not X-ray". If possible, a piece of X-ray film, a standard thermoluminescent dosimeter (TLD) or an optically stimulated luminescence (OSL) monitoring badge could be included in the package to indicate possible X-ray exposure.

8. Conclusion

For biodosimetry in radiation emergency medicine, great care must be taken in blood sample timing, anticoagulant selection and blood temperature control according to the type of anticoagulant used. It is also necessary to comply with international guidelines for blood shipment and transportation as human blood is an infectious biological specimen. In addition, as basic information on exposed patients is essential for dose estimation, it is necessary to standardize a recording format and establish an information sharing system with each institution. Furthermore, in order to prevent future accidents based

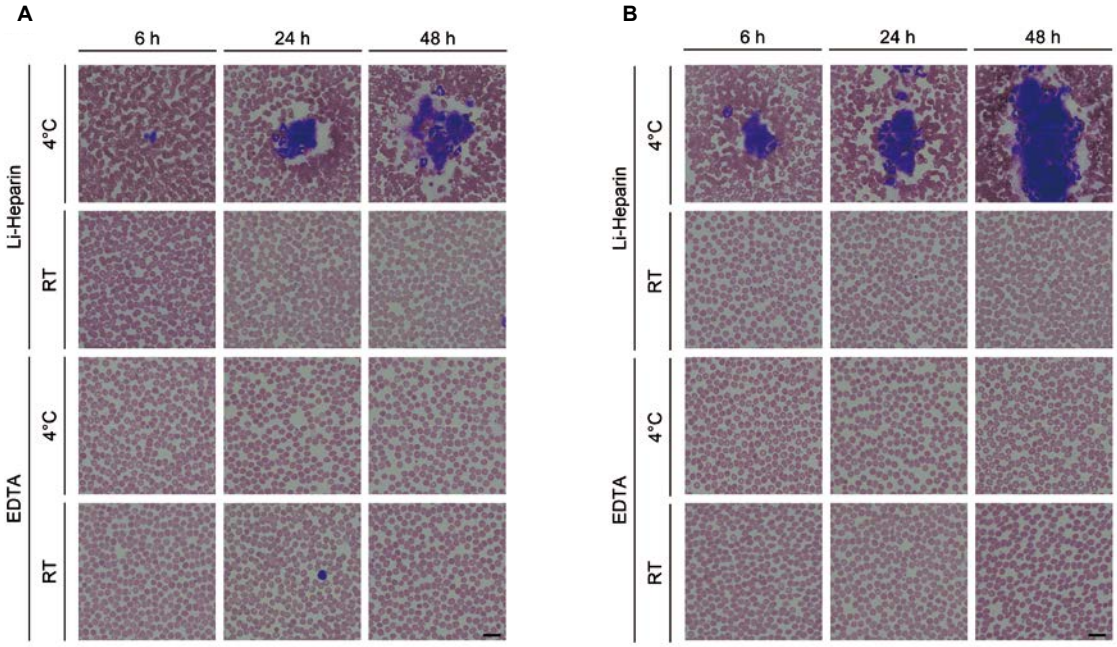

Fig. 2. Microscopic observations of May-Grünwald and Giemsa-stained blood smears. (A) 0 Gy-irradiated blood; (B) 3 Gy-irradiated blood. Scale bars represent 20 μm. Partial blood coagulation was only observed in Li-heparin blood stored at 4 ˚C (Data was modified from Fujishima *et al.* [18]).

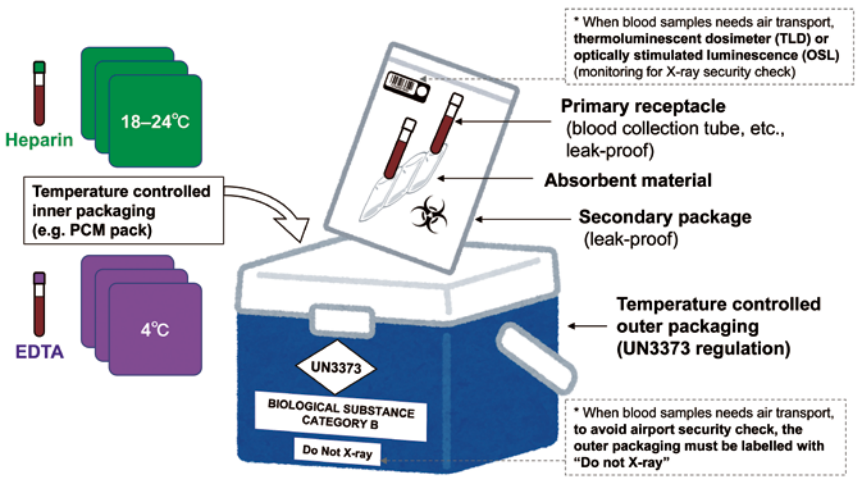

Fig. 3. Example of triple packaging used for blood transport for biodosimetry. Modified from WHO Guidance on regulations for the Transport of Infectious Substances[24].

on the lessons learned from the exposure accident, it is desirable to disclose information related to the accident, including the exposure dose. Please note that informed consent from exposed individual and employer (if needed) is required for information disclosure.

We hope that this article will help medical professionals involved in radiation emergency medicine understand biodosimetry.

Point 1. Time of blood collection

Blood collection period: 24 hours to 28 days

- If fatal high-dose exposure is suspected, blood should be drawn as soon as possible.
- For patients who require blood transfusion, draw blood before blood transfusion.
- Blood collection should not be forced if the patient's medical condition is serious.

Point 2. Acquisition of basic information and informed consent

- It is essential to collect and share basic information from exposed patients for dose assessment.
- It is necessary to obtain patient's consent to disclose the estimated dose.

Point 3. Anticoagulant

- Heparin is the preferred anticoagulant in biodosimetry.
- If EDTA blood is only available, be sure to indicate that blood was collected with EDTA.

Point 4. Blood volume

- If possible, collect 10 ml of blood from each patient.
- As blood leftover from blood tests can also be used for biodosimetry, store the leftover blood away from contamination.

Point 5. Blood storage

- Blood must be treated aseptically to avoid contamination.
- The storage temperature of heparin blood is 18 to 24 ℃.
  * Do not expose to cold conditions.
- EDTA blood can be stored at low temperatures of 4 ℃.
  * Do not freeze the blood.
- Blood is a potentially infectious substance. Please handle with care.

Point 6. Blood shipment

- Maintain recommended temperature using temperature controlled packaging.
- Blood is classified in biological substance Category B and transported in triple packaging according to UN and WHO standards (UN3373).
- To avoid airport security check, the outer packaging must be labelled with "Do not X-ray". A piece of X-ray film, a standard thermoluminescent dosimeter (TLD) or an optically stimulated luminescence (OSL) monitoring badge could also be included in the package.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Appendix

Sample Questionnaire

This questionnaire form has been modified from ISO 21243:2008

Exposure Information for Chromosome Aberration Analysis

(TO BE FILLED OUT BY THE REQUESTOR)

I, _____ (Name), born _____ (dd/mm/yy) consent to giving a blood sample for the purpose of estimating chromosome aberrations induced by exposure to ionizing radiation.

_____

Signature

Blood collection

Blood taken by:_____

Laboratory name:_____

Telephone number:_____ Fax:_____

E-mail: _____@_____

Date and time of blood taken:_____(dd/mm/yy) Type of anticoagulant:_____

Exposure Data:

1. ☐ Radiation Worker ☐ Non-Radiation Worker
2. Date and time of overexposure:_____(dd/mm/yy - time)
3. Place:_____ Company:_____
3. Brief description of overexposure:_____
4. ☐ Whole body exposure ☐ Partial body exposure ☐ Internal contamination

   Dose value:_____ Part of body:_____ Nuclide:_____

   Dose value:_____ Dose value:_____

   How was this dose value obtained?_____
5. Type of radiation: ☐ X-ray Energy:_____

   ☐ γ Origin:_____

   ☐ α Origin:_____

   ☐ Neutrons Origin:_____ Energy:_____

   ☐ Electrons Origin:_____ Energy:_____

Appendix. Sample questionnaire for chromosome aberration analysis (adapted from ISO 21243:2008)[13].

Patient Data

1. Age:_____ **Gender**:_____

2. Previous exposure through medical practice:

☐ Radiation therapy Date:_____ Part of body:_____

☐ X-ray diagnoses Date:_____ Part of body:_____

☐ Nuclear medicine Date:_____ Part of body:_____

2. Illness within the last 4 weeks before taking the blood sample:

_____

_____

3. ☐ **Intake of medication (fill in if applicable)**

Name of medication:_____ Dose:_____ Duration:_____

4. Smoker: ☐ No ☐ Yes Number / day_____

5. Alcohol intake: ☐ No ☐ Yes Volume / day_____

6. Other known diseases:

☐ HIV ☐ Hepatitis B ☐ Hepatitis C

Other diseases: _____

Results of chromosomal analysis to be sent to:

Name:_____

Address:_____

_____

Telephone number:_____ Fax:_____

E-mail: _____@_____

Appendix. Sample questionnaire for chromosome aberration analysis (adapted from ISO 21243:2008)[13].